Studies on Gregarines from Japan

I. *Cephaloidophora warekara* n. sp. and Two Other Gregarines from Crustaceans[1)]

By

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(With 2 Text-figures and 2 Plates)

During the study of the gregarines parasitic on crustaceans, the writer has noticed various species of the group Cephalina. Some of those members of the genus *Pyxinoides* from barnacles were reported in the former paper (K. Hoshide, 1968). In this paper two species of the genus *Cephaloidophora* and a species of the genus *Uradiophora* are described. The study was done in Hokkaido and in Yamaguchi prefecture, from summer 1968 till spring 1969.

The writer expresses his sincere appreciation to Professor Mayumi Yamada and also to Dr. Shoichi F. Sakagami for their kind and valuable guidance, criticism and advice throughout the course of this investigation.

Family Cephaloidophoridae Kamm 1922

Mawrodiadi (1908) created the genus *Cephaloidophora* for intestinal gregarines of barnacles, which had been found by Solger (1896) but unnamed. Mawrodiadi placed the genus *Cephaloidophora* in the family Stenophoridae for his animal was intracellular and had residual epimerite.

Sokolow (1911) listed the genus *Cephaloiodophora* in the family Stenophoridae too.

Léger and Duboscq (1907) found a gregarine from *Chthamalus* and named it *Frenzelina chthamali*. Into the genus *Frenzelina* they transferred various intestinal gregarines from other Crustacea. Thereafter they (1911) found that the two genera, *Cephaloidophora* and *Franzelina*, have the wholly identical characteristics and they recognized *Frenzelina* to be a synonym of *Cephaloidophora*.

Trégouboff (1912) transferred *Frenzelina chthamali* into the genus *Pyxinoides* because this species has no intracellular development.

Poisson (1920, 1921, 1924) found 4 new species of *Cephaloidophora* from Crustacea in France.

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Kamm (1922) published her opinion that the two genera, *Cephaloidophora* and *Stenophora*, should belong to a different family. The reason is as follows: The fact that in the former sporadins form the association early while in the latter sporadins are solitary till late, is the important points of difference. But the two genera have some common characters; intracellular development, rudimentary epimerite, ovoidal spores, cyst without sporeduct. She added three new species to the genus *Cephaloidophora* and created the new family Cephaloidophoridae which consists of one genus *Cephaloidophora*.

Ball (1937) studied on the gregarine in the gut of *Pachygrapsus marmoratus* and erected a new genus *Carcinoecetes* into which he put *Cephaloidophora conformis* (Diesing) Léger and Duboscq. He explained that the genus *Cephaloidophora* has sporocysts discharged in chain but *Carcinoecetes* sporocysts not in chain. Thereafter he recognized that the *Carcinoecetes* is the synonym of the *Porosporidae* (1951) and reported two new species of *Cephaloidophora* (1963).

Henry (1938) examined many gregarines of barnacles in America and gave out descriptions about *Cephaloidophora communis* and other 6 species of gregaines in which 2 new members of genus *Cephaloidophora* were comprised.

H. Hoshide (1951, 1957) reported 12 species of gregarines from Crustacea in Japan, which included one known and 6 new species of *Cephaloidophora*. He did not discriminate between *Carcinoecetes* and *Porospora*.

Théodoridès (1961, 62, 64, 65) confirmed the distinction points between the families Cephaloidophoridae and Porosporidae: the members of the former have always the protomerite in the satellite and the distinct septum is as visible as the epicyte between the satellite and primite. He published many papers in which he reported 5 new species not yet certified and several known ones of the genus *Cephaloidophora*.

1. *Cephaloidophora uarekara* n. sp.

(Fig. 1 A-C, Pl. II, Fig. 1-2)

Host: *Caprella* sp.
Habitat: Intestine.
Locality: Akkeshi (Hokkaido)

The host *Caprella* sp. is comparatively large in size, attaining 25 mm in length. They are easily collected among seaweeds at Aikappu point in Akkeshi Bay, where the seaweeds grow on rocks always washed with rather rough waves. The parasitic rate of this gregarine on the host is fairly high. More than 60% of the hosts examined were parasitized by this species, in June of 1968.

Sporadins: The sporadins are biassociative, but sometimes they associate in three or four; at the same time 2 or 3 individuals attach at the end of one primite. They are ovoidal or short cylindrical in shape. The maximum recorded length for an association was 220 μ.

The average size of the body is as follows; all dimensions are expressed in microns:

TL 83, LP 24, LD 59, WP 30, WD 39, tl 84, lp 21, ld 63, wp 30, wd 36.

Ratio; LP: TL=1:3.5, WP:WD=1:1.3, lp:tl=1:4.0, wp:wd=1:1.2.

(Primite): The protomerite is subglobular in shape, and is always rather wide than long, widest at the central portion. There is a lens-shaped, transparent projection at the anterior end of the protomerite.

The posterior end of the protomerite falls downward and projects into the deutomerite like a low knoll at the center of the septum.

The septum between protomerite and deutomerite is clear but the constriction here is not so deep.

The deutomerite is ovoidal, widest at a little behind the middle, thence it tapers very gradually to the posterior portion and ends in a broadly rounded extremity. There is a shallow depression at the center of this terminal surface, into which the anterior knob-like projection of the satellite protomerite just fits. In many specimens a large concavity of the body wall occurs on one side of the body.

Table 1.

Total length of association	121	131	157	204	164
Primite					
TL	66	68	81	101	73
LP	15	20	30	28	20
LD	50	48	51	73	53
WP	23	25	30	33	30
WD	20	38	43	48	43
Ratio					
LP:TL	1:4.4	1:3.4	1:2.7	1:3.6	1:3.7
WP:WD	1:0.9	1:1.5	1:1.4	1:1.5	1:1.4
Satellite					
tl	55	63	76	103	91
lp	13	20	23	25	18
ld	43	43	53	78	73
wp	20	25	30	35	25
wd	20	30	38	50	30
Ratio					
lp:tl	1:4.2	1:3.2	1:3.3	1:4.1	1:5.1
wp:wd	1:1.0	1:1.2	1:1.3	1:1.4	1:1.2

(Satellite): The interlocking device between the primite and satellite is week, because they join feebly each other at the septum. The protomerite of satellite is nearly the same as that of the primite in shape but the former is usually a little shorter than the latter. There is a lens-shaped projection as that of the primite at the anterior end of the protomerite. The constriction at the septum is shallow.

The deutomerite is ovoidal and is almost alike that of the primite except the appearance of the posterior extremity. A complex concave is visible at the center of the terminal surface and give rise several deep furrows dividing the surface into some lobes around the concave.

Both endoplasms of the protomerite and the deutomerite are very different. The protomerite granules are much larger than those of the deutomerite and scatter sparsely. The endoplasm of deutomerite is dense, being light brown in transmitted light. The granules in the endoplasm are fine and rather homogenous.

The nucleus is spherical and visible in vivo. It measures 14 μ on an average in diameter, containing a comparatively large spherical nucleolus, 5–8 μ in diameter.

Remarks: Frenzel (1855) found a gregarine from *Caprella* sp. in Naples and named it *Gregarina caprellae*. The Frenzel's species is decidedly different from the present species in size and shape of the sporadin.

This species resembles *Cephaloidophora delphinia* (Watson) Kamm but in the following points there are differences between them: 1) In *C. delphinia*, the apex of the protomerite is slightly papillated, but in this species there is a large lens-shaped projection at the apex of the protomerite. 2) In *C. delphinia*, the deutomerite is broad, measuring more than 60 μ in width but in this species it is less than 50μ. 3) In *C. delphinia*, the posterior end of satellite deutomerite terminates in a broadly rounded extremity but in this species there is a peculiar concavity.

This species is also similar in size and in ratio of the body to *Cephaloidophora olivia* (Watson) Kamm but the former is easily separated from *C. olivia* in the appearance of endoplasm; the latter has dark brown endoplasm in the deutomerite and that in the protomerite but the former has light brown in the deutomerite and lighter in the protomerite, moreover the protomerite granules are very large in this species.

When one sets this species against any other members of the genus *Cephaloidophora*, this has some peculiar characteristics as follows:

1) There is a large and fairly deep concavity at the posterior terminal surface of the satellite deutomerite. Then several deep furrows and lobes are found around the concavity.

2) The endoplasms of protomerite and deutomerite are very different; the granules in the former are much larger than those in the latter.

3) The lens-shaped clear projection at the anterior end of protomerite is very large and it shoots out from the protomerite.

2. *Cephaloidophora setoutiensis minor*, n. subsp.

(Fig. 1 D–H, Pl. II, Fig. 3–6)

Host: *Orchestia ochotensis* Brandt
Habitat: Intestine.
Locality: Akkeshi (Hokkaido).

The material was taken at Akkeshi Bay, in June, 1968. The host is a large sand flea which is easily captured under stones or among seeweeds lying on the beach. This host was found very frequently infected with this parasite, and more than 50% of 100 individuals of this sand flex exmained were parasitized during the summer season.

Sporadins: The sporadins are biassociative. The maximum length observed for an association was 95μ. The largest sporadin was 50μ long and 25μ wide. The sporadins are elongated ovoidal to somewhat cylindrical in shape.

The average size of the body is as follows; all dimensions are expressed in microns:

TL 42, LP 12, LD30, WP 14, WD 18, tl 37, lp 10, ld 27, wp 13, wd 17.

Ratio: LP:TL=1:3.4, WP:WD=1:1.3, lp:tl=1:3.6, wp:wd=1:1.3.

(Primite): The body is generally cylindrical in shape. When the sporadins are mature, they fatten a little and show an ovoidal shape.

The protomerite is hemispherical, widest in the middle and usually rather wide than long. A lens-shaped transparent projection is observed at the anterior end of the protomerite. The projection measures about 8μ in width and 3μ in length.

The constriction at the septum is slight. The deutomerite is ellipsoidal to cylindrical in shape and it broadens gradually from the septum to the middle, the part of which is widest in most cases. But sometimes the widest position lies at other part of the deutomerite, a little above or below the middle or near the septum. From the widest region the deutomerite gradually tapers to a broad and truncated posterior end.

(Satellite): In almost all the cases the satellite is a little smaller and more slender than the primite, but the satellite is the same as the primite in the shape of body. The protomerite is slightly depressed at the top into which the posterior

Table 2.

Total length of association	68	68	73	68	90
Primite					
TL	38	35	38	38	45
LP	10	10	10	10	15
LD	28	25	28	28	30
WP	13	13	13	15	15
WD	13	15	15	18	20
Ratio					
LP:TL	1:3.8	1:3.5	1:3.8	1:3.8	1:3.0
WP:WD	1:1.0	1:1.2	1:1.2	1:1.2	1:1.3
Satellite					
tl	30	33	35	30	45
lp	8	8	8	8	13
ld	23	25	23	23	33
wp	13	13	13	13	15
wd	15	15	15	15	20
Ratio					
lp:tl	1:3.8	1:4.1	1:4.4	1:3.8	1:3.5
wp:wd	1:1.2	1:1.2	1:1.2	1:1.2	1:1.3

end of the primite is fixed. The coupling device is well developed and they connect firmly each other. A clear lens-shaped projection is also visible at the top of the satellite.

There is a shallow constriction at the septum. The deutomerite is almost the same as that of primite. It is widest at middle and tapers to the posterior end terminating in a broadly rounded or flattened extremity.

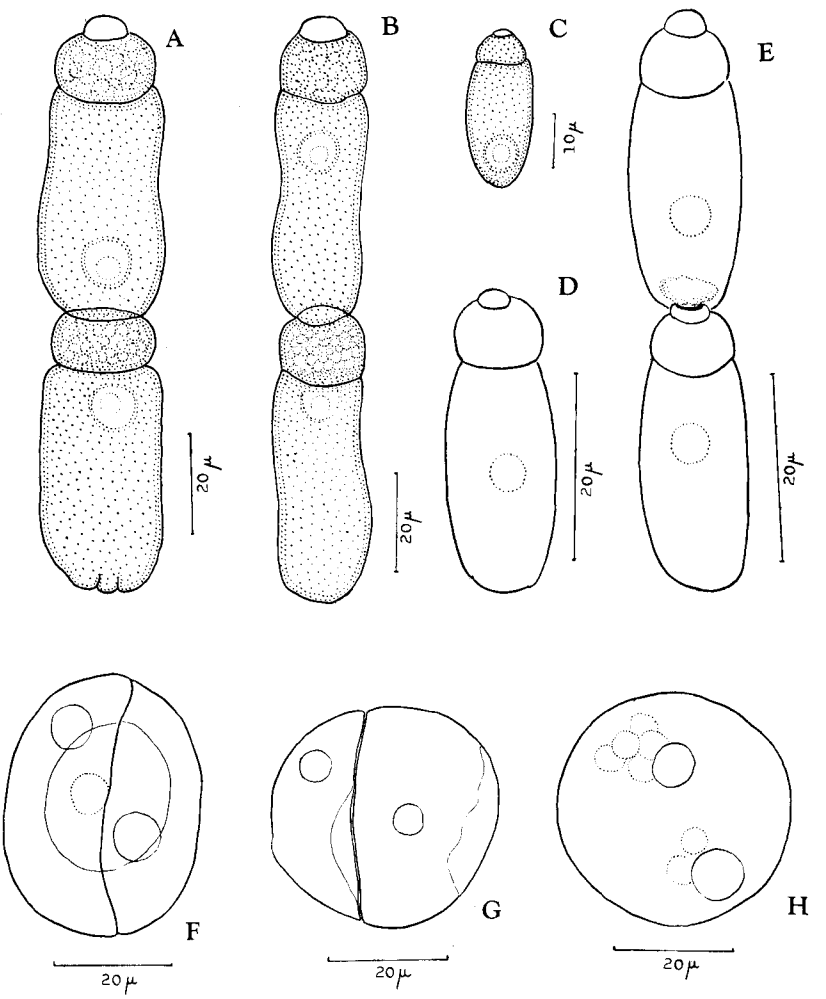

Fig. 1. A–C. *Cephaloidophora warekara* n. sp., A. Large adult association. B. Another adult association. C. Small trophozoite. D–H. *Cephaloidophora setoutiensis minor* n. subsp. D. Large trophozoite. E. Mature association. F-H. Cyst formation, early stage to developed stage.

The ectoplasm is rather thin except the anterior region of the protomerite. The endoplasm is brown in transmitted light. The granules contained in the protomerite are somewhat coarser than those in the deutomerite.

The nucleus is spherical and well visible in vivo. It lies generally about in the middle of the deutomerite and it measures 5–8 μ in diameter. A spherical nucleolus is visible in it.

Cyst and Spore: At the beginning of cyst formation, the associated couple begin to bend inwards and revolve around an axis. They finally come to lie side by side and fasten them firmly each other to form a sphere covered with a common transparent envelope. In course of this cyst-formation one can see a fine groove between two individuals and as the time goes by the groove becomes more visible.

The cysts are spherical or somewhat ellipsoidal in shape and measure 33–40 μ in diameter.

Spores were not observed.

Remarks: This subspecies is similar in the body shape to *Cephaloidophora orchestiae* Poisson et Remy, but the former is only 50μ in maximum body length while the latter 140μ.

The present subspecies resembles *Cephaloidophora poissoni* Theodorides in the body size but the former differs from the latter in the shape of the body; the former is more or less slender than the latter; the primite and satellite are almost the same in shape in the former and they are different in the latter. In *C. poissoni* the posterior end of satellite is rather pointed whereas in this species is broadly rounded or flattened.

Though this subspecies closely resembles *Cephaloidophora setoutiensis* H. Hoshide from Yamaguchi pref. which is the parasite of *Orchestia platensis*, they should be separated because the former is a little smaller than the latter in the stage of mature sporadins–the maximum association of this subspecies is 95μ but that of *C. setoutiensis* from Yamaguchi pref. 140 μ, the maximum length of sporadin is 50μ in this subspecies but that is 80μ in *C. setoutiensis* from Yamaguchi pref. – : the former has the average ratio of LP:TL=1:3.4, WP:WD=1:1.3, lp: tl= 1:3.6, wp: wd=1:1.3, whereas the latter has LP:TL=1:3.3, WP:WD=1:1.3, lp: tl=1:3.1 wp:wd=1:1.3.

The difference between two forms is not so striking as to divide them into a different species, then the writer will situate the present one as new subspecies; *Cephaloidophora setoutiensis minor* n. subsp.

Family Gregarinidae Labbé 1899

3. *Uradiophora cuenoti* Mercier 1911

(Fig. 2, Pl. III, Fig. 1–7)

Host: *Neocardina denticulta* (De Haan)
Habitat: Intestine.

Locality: Yamaguchi, Tabuse, Hikari (Yamaguchi pref.).

The host of this parasite is a common fresh-water shrimp inhabiting streams, ponds and ditches around paddy fields, of the western areas of Japan. The parasite was found very commonly in the intestine of the shrimp.

Sporadins: The sporadins are biassociative. The body is elongate cylindrical in shape. The longest association observed was 550μ, and the maximum length of sporadin is under 300μ. The largest sporadin in primite observed was 265μ in length and 23μ in width.

The average size of the body is as follows; all dimensions are expressed in microns:

TL 175, LP 9, LD 165, WP 13, WD 18, tl 243, lp 9, ld 234, wp 13, wd 20.

Ratio; LP:TL=1:18, WP:WD=1:1.4, lp: tl=1:26, wp:wd=1:1.7.

(Primite): The protomerite is subglobular slightly wider than its length and is widest in the middle. It is rounded at the apex. There is a fairly deep constriction at the septum. The deutomerite is elongated, slender, cylindrical, widening very gradually from the septum and attaining the greatest width through the middle or some distance below the middle. It terminates in a broadly rounded posterior extremity. The width of the deutomerite, however, is almost of even width throughout the length.

The epimerite, which persists in well-developed free individuals, consists of a flattened pen-shaped crown with a small ovoidal papilla at its apex inserted into

Table 3,

Total length of association	498	495	435	390	270
Primite					
TL	254	185	200	145	90
LP	12	10	11	9	7
LD	242	175	189	136	83
WP	20	10	15	13	8
WD	26	16	20	20	10
Ratio					
LP:TL	1:21.2	1:18.5	1:18.2	1:16.1	1:12.9
WP:WD	1: 1.3	1: 1.6	1: 1.3	1: 1.5	1: 1.2
Satellite					
tl	244	310	235	245	180
lp	8	12	8	12	7
ld	236	298	227	235	173
wp	13	13	12	12	13
wd	21	20	20	21	18
Ratio					
lp:tl	1:30.5	1:25.8	1:29.4	1:20.3	1:28.6
wp:wd	1: 1.6	1: 1.5	1: 1.7	1: 1.8	1: 1.4

the epithelial cell of the host gut. A fine canal runs through the center of the basal crown, connecting the apical papilla with the protomerite.

(Satellite): The apex of the satellite projects conically and fits into a depression in the deutomerite of the primite. So the interlocking device between primite and satellite is well developed. The protomerite is subglobular. It is as wide as long or is a little wider than its length. The protomerite is widest at the point a short distance above the septum. There is a slight constriction at the septum. The deutomerite is long, slender cylindrical, widening very gradually form the septum and is widest usually at some distance below the middle where the nucleus generally situates. It is of even width throughout and only a little wider than the primite is. There is an atrophied appendix which is stuck to the posterior end of the deutomerite. The appendix measures $35 \times 10\mu$ in large specimens, and is ellipsoidal in shape.

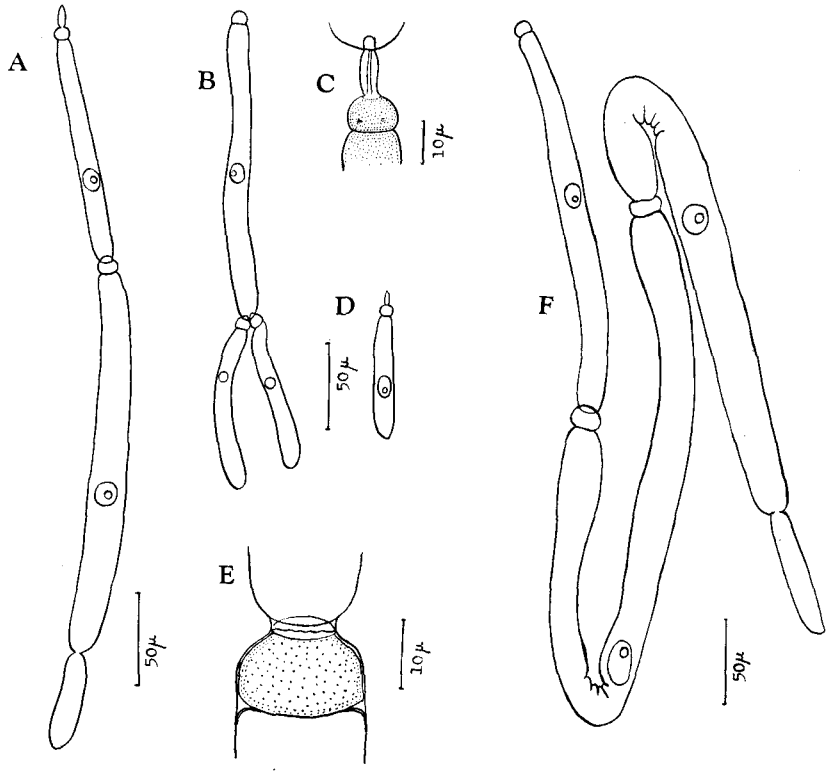

Fig. 2. *Uradiophora cuenoti* Mercier. A. Associated sporonts. B. Two satellites associated with one primite. C. Epimerite. D. Trophozoite. E. Interlocking region of association. F. Assocaition of three sporonts.

In colour, the body is light brown, of equal density both in protomerite and deutomerite: the protoplasm is dense and homogeneous. The small anterior region of the protomerite and the epimerite are transparent. The epicyte is thin and of even width throughout.

The nucleus is spherical. The position of the nucleus in the deutomerite is variable, most often it is situated in the widest portion of the deutomerite. It contains a spherical nucleolus in it.

Movement: two types of movement were observed. A rapid gliding motion at the rate of 7μ per second was very commonly observed.

Cyst and Spore: The cysts collected from the excreta or from the cadual fins, where they are often found, are ellipsoidal or ovoidal in shape and have an average measurement of cysts $60 \times 25\,\mu$. The spores are hemispherical and measure 4–5μ in diameter.

Remarks: The present species in Japan and *Uradiophora cuenoti* Mercier in France have almost the same characteristics of their shape, size, ratio of body parts, nucleus and appendix of deutomerite. The writer reports Yamaguchi prefecture of Japan as a new locality for *Uradiophora cuenoti* Mercier.

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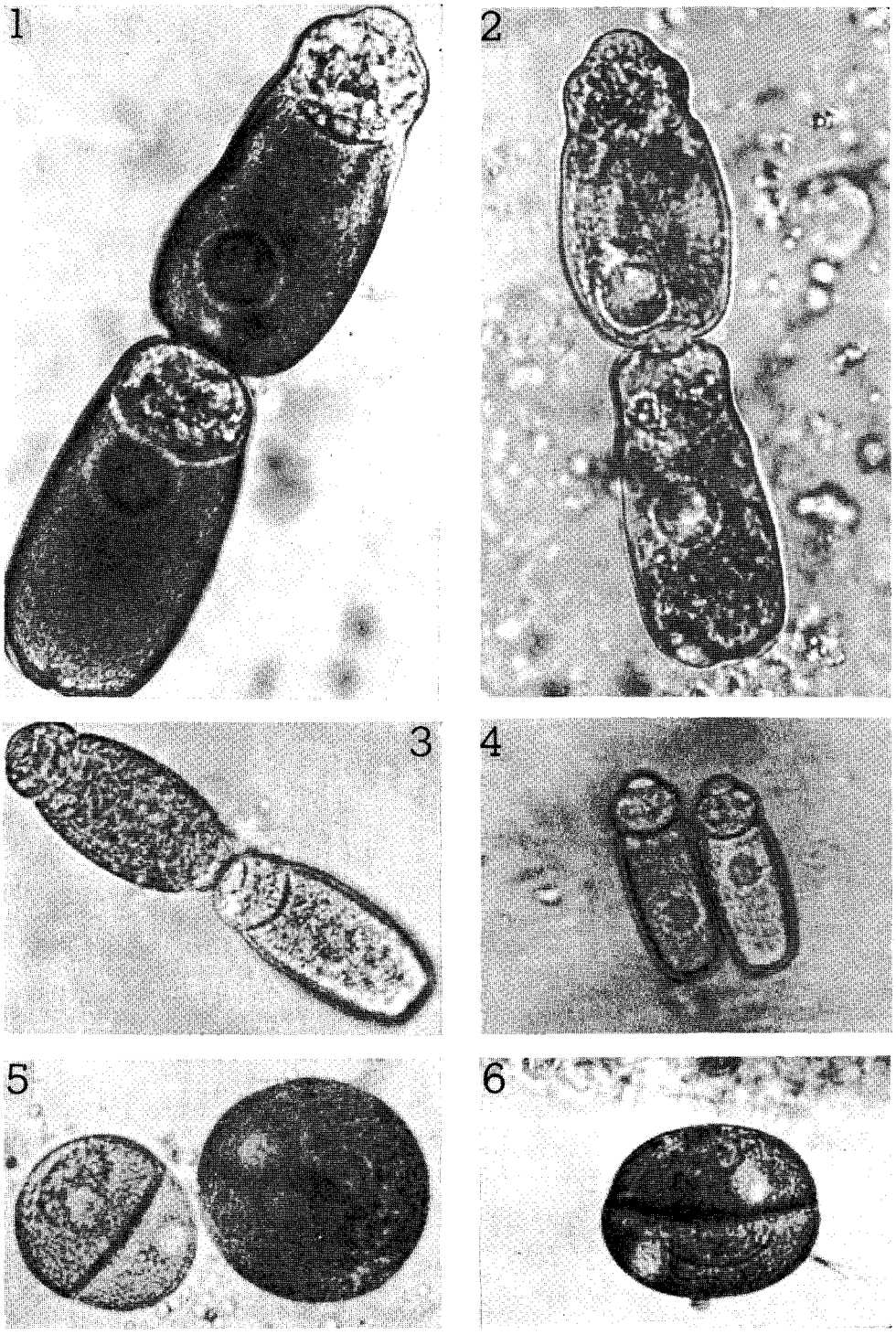
Palte II

Figs. 1–2: *Cephaloidophora warekara* n. sp.
1. Associated sporadins. Endocyte of protomerite and of deutomerite different in quality. × 590. 2. Associated sporadins. Spherical nucleus with one nucleolus. × 590.
Figs. 3–6: *Cephaloidophora setoutiensis minor* n. subsp.
3. Mature association. × 680. 4. Two trophozoties. × 680. 5. Two cysts, left one early stage and right one developed stage of cyst formation. × 680. 6. Two sporadins attached to the body side of each other and just completed their cyst formation. × 680.

Palte III

Figs. 1–7: *Uradiophora cuenoti* Mercier
1. Associated sporadins. × 150. 2. Posterior part of satellite with small appendix. × 300. 3. Many sporadins attached to intestinal wall. × 95. 4. Sporadine attached to host cells. × 95. 5. Cyst. × 310. 6. A breaking cyst and spores comming out. 7. Spores in a cyst more magnified. × 1700.



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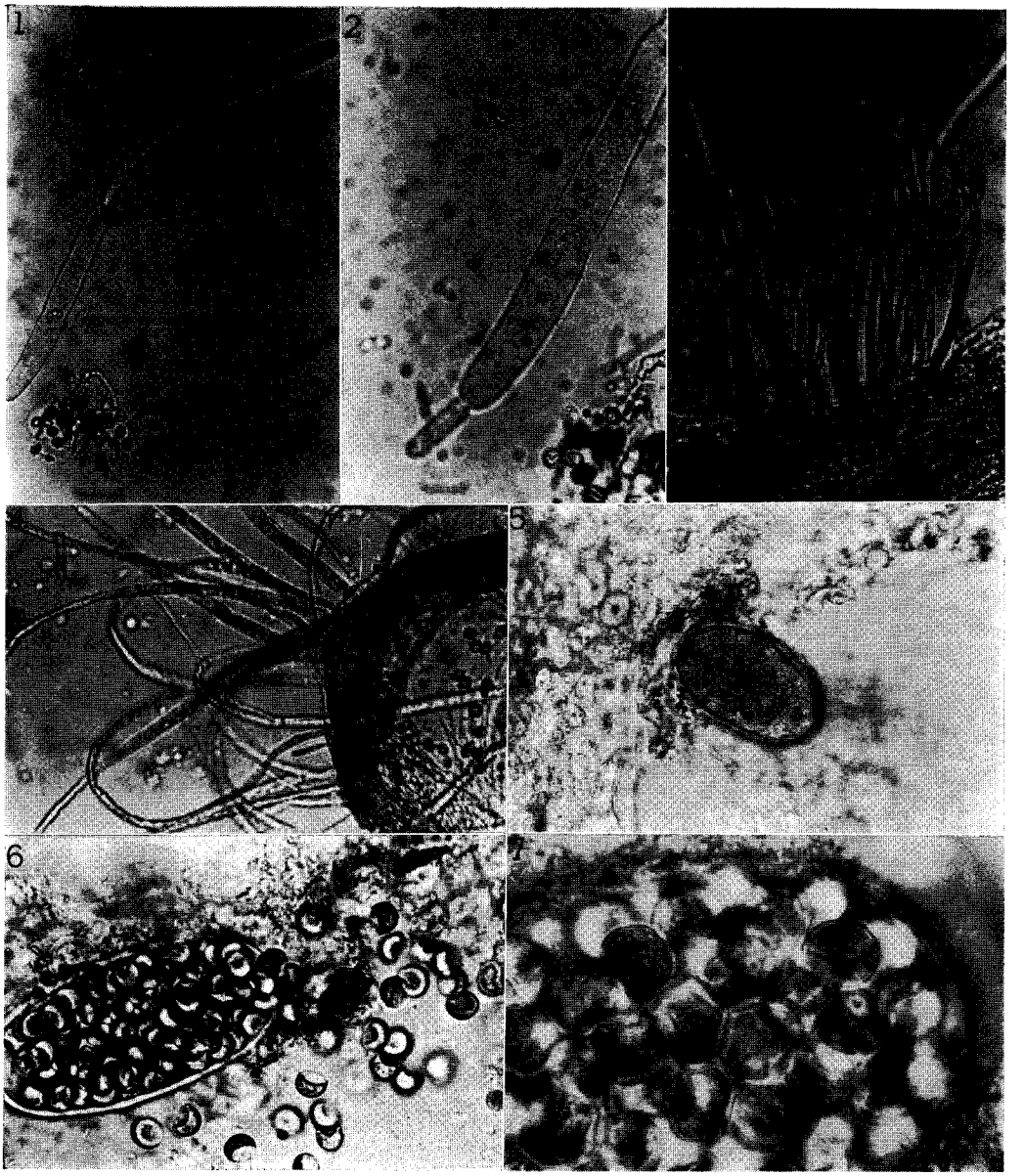

K. Hoshide: Cephaline Gregarines from Some Crustaceans